

but they spontaneously migrate to the SLG areas and eventually form an ordered interconnected neuron pattern perfectly superimposed to the pattern design. Surface functionalization was then more effective on the SLG, and resulted in notably aligned neural network. The described technique could be considered a valuable candidate to realize a new generation of highly specialized biosensors. To gain further insight into the preferential positioning of neurons onto SLG, the distribution of focal adhesion proteins on the patterned SLG was investigated; Stochastic Optical Reconstruction Microscopy (STORM) was employed in order to localize the cellular components for focal adhesion. Super resolution imaging qualitatively confirms that the distribution of vinculin molecules tagged with Alexa 647 has more affinity towards the SLG regions compared to the ablated ones.

1 Lorenzoni et al, Scientific Reports 3:1954, DOI:10.1038/srep01954.

1063-Pos Board B818

Cell-Permissive Protein-Resistant Substrates for Interrogating Neuronal Guidance Cues

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Current in vitro tools for evaluating neuronal guidance cues suffer from several drawbacks, including difficult substrate preparation and hard-to-interpret results. Microcontact printing of an alkanethiol self-assembled monolayer (SAM) provides a robust method for producing high-resolution protein patterns by protein adsorption from solution. However, cell and protein resistant glycol terminated monolayers are typically employed in the background, which prevents the evaluation of potential neuronal guidance cues. We have developed zwitterionic background monolayers that are protein-resistant, but remain cell-permissive. Using surface plasmon resonance imaging (SPRi) and cell-culture studies, we have demonstrated that these zwitterionic monolayers provide well-defined, non-receptor mediate cellular attachment through interactions with cell-surface glycosylation. Exploiting these properties, we have created a monolayer based stripe assay, where the interactions between neurons (cell bodies and neurites) and extracellular matrix (ECM) proteins or guidance cues may be observed and quantified. This system goes beyond current technologies, such as direct protein patterning and microfluidics, and is even capable of evaluating neuronal response to ECM protein, such as laminin.

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Conductive Milieu on Cellular Electromechanics

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Cellular polarity and alignment are significant biophysical factors in tissue architectures and tissue-to-organ level functions. Multiple biophysical contributors are addressed as cellular alignment factors in mechanical, biochemical and electrical aspects; however, passive electric conductance has not been discussed. Herein, we report the spontaneous alignment of cardiomyocytes to pre-defined electric conductivity environments as example. Two to three days after passaging iPSCs-derived cardiomyocytes on thin gold strips (i.e., 10-nm thickness, 10s- μ m width and 10s- μ m period) patterned on a nonconductive substrate (i.e., glass), the most single-cell cardiomyocytes adhere on the nonconductive area and align themselves parallel to the conductive pattern, without any external stimuli. From control experiments, we can exclude any mechanical cues as the reasons of the spontaneous alignment, such as surface groove and mechanical stiffness. Currently, we hypothesize diamagnetic effect or Fröhlich electromagnetic effect as the physiological response of cardiomyocyte, which may be induced from the electric field coupling between cardiomyocytes and the gold pattern. Along with the further understanding, this observation will highlight passive electric elements as important biophysical aspects since many cellular components possess a level of electric properties.

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Contractility of Neonatal Cardiomyocytes is Altered with Different Densities of Laminin Covalently Attached to Microposts

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Heart disease is the number one killer in the USA. The collective contractility of the muscle cells of the myocardium - cardiomyocytes - generates the necessary force for the function of a healthy beating heart. Laminin interacts in vivo with cardiomyocytes. Changes in the extracellular concentration and organization of laminin relate to different types of heart disease. Arrays of polydimethylsiloxane (PDMS) microposts measure forces generated by adhesive mammalian cells and were here used to characterize the contractility of single

neonatal cardiomyocytes. We used two types of organosilanes to bind laminin to the surface of PDMS microposts: 3-glycidioxypropyltrimethoxysilane and 3-aminopropyltriethoxysilane. We acquired videos of contracting cardiomyocytes at two different days after cells started to beat and functionally characterized the contractility of single cells. More specifically, we calculated generated forces, beating rate, time of contractions and speeds of contraction and relaxation. These parameters varied in time as a function of organosilane surface stability and cardiomyocyte biological changes when cultured in vitro. Higher forces are generated by cardiomyocytes cultured on laminin covalently attached to PDMS microposts relative to laminin physisorbed to oxidized PDMS. We obtained higher laminin density with 3-glycidioxypropyltrimethoxysilane, which correlated to higher generated forces. We also observed higher beating rate at the day 1 and a considerable decrease at day 2. Compared to 3-glycidioxypropyltrimethoxysilane, higher stability of laminin covalent attachment was observed with 3-aminopropyltriethoxysilane. The beating rate and speeds of contraction and relaxation increased and time of contractions decreased at day 2 for neonatal cardiomyocytes cultured on these PDMS micropost surfaces. Our results shed light on the potential of in vitro biomechanical systems to model extracellular disease conditions of heart pathologies. Future work will test the contractility of cardiomyocytes with mutations known to originate cardiomyopathies.

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Flow Injection of DNA in Nanopores : Direct Optical Visualization of a Pressure Threshold

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In the regime of pores smaller than the radius of gyration, the flow injection of polymers and biopolymers exhibit a flow threshold independent of the pore and of the polymer itself. We have developed a new combination of near field optics (zero mode wave guide) and image analysis in order to revisit this phenomenon. Working at constant pressure we are able to control and observe directly the transport of individual biomolecules with a time resolution of 5 ms. In the case of DNA, we show that the forced transport through the pore can be described as an energetic barrier only dependent on the injected flow. Further application to biological systems of this barrier measurement will also be discussed.

1067-Pos Board B822

Designing Hydrophobic Gates into Biomimetic Nanopores

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The use of nanopores is fast being a major scientific tool in molecular analysis and detection due to their ability to detect polynucleotides, proteins and small molecules. Biomimetic modelling of pores allows for a specific function to be incorporated into the molecular structure of the nanopore, based on amino acid motifs found in existing protein structures.

An initial beta barrel model was built computationally, based on the transmembrane domain of 14 stranded beta-barrel pore, alpha-hemolysin. Hydrophobic and hydrophilic residues were built in a specific arrangement within the structure to replicate an hourglass shape cavity with a central constriction. From this, pore conductions were observed via Molecular Dynamics (MD) and selected models were transformed into hybrid pores in which the location of hydrophobic residues differed to give constricting regions surrounded by hydrophilic residues. From All Atom MD simulations, a hydrophobic gating mechanism has been established within these toy models with intermittent water currents through the pore giving an insight into possible biomimetic motifs which could be biochemically integrated into the wild type protein.

1068-Pos Board B823

Developing a Broadband Amplifier for Analysis of DNA Structural and Molecular Characteristics

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The recent emergence of DNA-based diagnostics increases the need for rapid DNA sequencing technologies. One method to achieve this is to pass DNA through a nanopore, recording the trans-membrane current with a low-noise current amplifier. The challenge presented in this method is that the bandwidth of commercially available current amplifiers is limited to 100kHz, which is not